

REMARKS

These remarks and the above amendments are responsive to the Office action dated June 3, 2004. Claims 94-105 and 108-115 are pending in the application. In the Office action, the Examiner rejected claim 94 under 35 U.S.C. § 112, first paragraph, as being insufficiently enabled, and claims 94-101, 108, 110, and 112-115 as being obvious under 35 U.S.C. § 103(a). In addition, the Examiner objected to claims 102-105, 109, and 111, but indicated that they would be allowable if rewritten to include all of the limitations of the base claim (claim 94) and any intervening claims, and to overcome the rejection(s) of the base claim under 35 U.S.C. § 112.

Applicants traverse the rejections. Applicants contend that the rejected claims are both fully enabled and nonobvious. Nevertheless, to reduce the number of issues under consideration, and to expedite the issuance of a patent, applicants have amended independent claim 94 to specify an enzyme performing a phosphate modification. Moreover, applicants have presented data and arguments showing that the claimed invention has significant unexpected benefits. These benefits, which include a substantial increase in luminescence intensity upon phosphate-gallium binding, as opposed to a substantial decrease or quenching in luminescence intensity upon phosphate-iron binding, are neither taught nor suggested by the prior art. Moreover, these benefits may provide truly dramatic differences in the timing and sensitivity of associated assays. Accordingly, applicants respectfully request reconsideration of the rejected claims and prompt issuance of a notice of allowance.

I. **Claim Rejections – 35 U.S.C. § 112**

The Examiner rejected claim 94 in the Office action under 35 U.S.C. § 112, first paragraph, for being insufficiently enabled. In particular, the Examiner asserted that applicants have not provided sufficient enablement in the specification for modifications other than phosphate modifications. Applicants traverse this rejection. Applicants believe that modifications other than phosphate modifications are enabled by the specification. Nevertheless, to reduce the number of issues under consideration, and to expedite the issuance of a patent from the present application, applicants have amended claim 94 to recite, in part, "a method of detecting the activity of an enzyme that **performs a phosphate modification**" (phrase added by amendment shown in bold and underline). Applicants further have amended claims 97 and 99-101, and canceled claim 98, to address formal issues created by the amendment of claim 94. Applicants believe claim 94, as amended, overcomes the rejection under 35 U.S.C. § 112, first paragraph. Thus, for at least this reason, applicants respectfully request withdrawal of the rejection of claim 94 under 35 U.S.C. § 112, first paragraph.

II. **Claim Rejections – 35 U.S.C. § 103**

The Examiner rejected independent claim 94 and dependent claims 95-101, 108, 110, and 112-115 as being unpatentable over Nikiforov (U.S. Patent No. 6,472,141) in view of Posewitz et al. ("Posewitz") (Anal. Chem. 7:2883-92 (1999)). Applicants traverse these rejections, at least because the claimed invention has unexpected benefits. These benefits, which include a substantial increase in luminescence intensity upon phosphate-gallium binding, as opposed to a substantial decrease or quenching in luminescence intensity upon phosphate-iron binding, are neither taught nor suggested

by either reference, alone or in combination, or any other reference of record. Moreover, these benefits may provide truly dramatic (potentially 100-fold) differences in the timing and sensitivity of associated assays.

II.A. Summary of the Examiner's Rejection for Obviousness

The Examiner rejected the above-identified claims under 35 U.S.C. § 103(a) for obviousness using a combination of Nikiforov and Posewitz. The Examiner's grounds for obviousness are summarized here.

The Examiner relied on Nikiforov as the primary reference for rejecting the above-identified claims.¹ The Examiner asserted that Nikiforov teaches all but one limitation of these claims. In particular, the Examiner asserted that Nikiforov teaches (1) a fluorescence polarization assay to determine enzymatic activity of a phosphatase or kinase on a polypeptide substrate, (2) steps of contacting the substrate with enzyme and then with a metal ion (Fe^{+3} , Ca^{+2} , Ni^{+2} , or Zn^{+2}), and (3) a step of detecting luminescence polarization from the sample. However, the Examiner admitted that Nikiforov does not teach or suggest the use of a binding partner including gallium, as recited in these claims.

The Examiner relied on Posewitz as a secondary reference for combination with Nikiforov, to try to provide a teaching for the use of gallium.² In particular, the Examiner asserted that Posewitz teaches using gallium in place of iron for phosphopeptide purification. The Examiner concluded that it therefore would have been obvious to one having ordinary skill in the art at the time the invention was made to replace the conventional metal ions (particularly iron) for capturing phosphopeptides as taught by

¹ Office Action (June 3, 2004), page 4, first paragraph.

² Id. at page 4, second paragraph to page 5, first full paragraph.

Nikiforov with the gallium ion taught by Posewitz, with a reasonable expectation of success. In support of this position, the Examiner appears to suggest that Posewitz provides two motivations for this replacement: (1) an assertion that iron and gallium ions are known to possess similar behavior in iron binding proteins (for example, gallium ions may be substituted for iron ions to study the mechanism of iron binding proteins); and (2) an assertion that gallium ions are more selective and efficient than iron ions for purification of phosphopeptides.

II.B. Summary of Applicants' Evidence for Unexpected Benefits

Applicants already have submitted evidence of unexpected benefits of gallium over iron.³ In particular, in their response to the previous Office action on this application, applicants provided a declaration from Dr. Richard Sportsman, an expert on luminescence polarization assays and one of the inventors on the current application.⁴ The declaration described at least three significant unexpected benefits of gallium as binding partner in luminescence polarization assays:

- First, gallium, in contrast to iron, enhances intensity, instead of quenching intensity, after associating with luminescent assay components. Consequently, assays employing gallium can be performed much more quickly and with much less statistical noise than assays employing iron.
- Second, assays employing gallium, in contrast to assays employing iron, have a much greater dynamic range of polarization. Consequently, assays employing gallium are much more robust and easy to perform than assays employing iron, if the latter can be performed at all.
- Third, assays employing gallium to bind product can better distinguish the existence of product in a mixture of substrate and product, relative to iron, since product bound to gallium will contribute more rather than less to the total polarization.

³ Response to Office Action (April 5, 2004).

⁴ Id. at Exhibit A.

Accordingly, in the previous response to Office action, applicants contended (and still contend) that these unexpected benefits rebut any assertion of *prima facie* obviousness offered by the Examiner.

None of these benefits is taught or suggested by Nikiforov, Posewitz, or any other prior art reference of record.

II.C. Examiner's Response to Applicants' Unexpected Benefits

In the current Office action, the Examiner stated that applicants' arguments for the unexpected benefits of gallium were not persuasive.⁵ The Examiner justified this position by citing the following passage from Posewitz:

“It is therefore not surprising that it [Ga] mimics the proven ability of Fe to bind phosphorylated peptides. In addition, Ga must possess certain unique features to provide more selective retention of phosphorylated peptides”⁶

Based on this statement in Posewitz, the Examiner apparently then concluded that each of applicants' benefits produced by replacing iron with gallium would be expected because of gallium's “unique features.”

II.D. Applicants' Rebuttal to the Examiner's Response

Applicants strongly disagree with the Examiner's position that any of the benefits of gallium presented by applicants would be expected based on the teachings of Posewitz. In particular, applicants believe that nothing in Posewitz even arguably teaches or suggests that gallium has an ability to provide any of the above-identified benefits: (1) enhanced luminescence intensity instead of quenching, (2) a much greater dynamic range of polarization, or (3) more sensitive recognition of product in a polarization assay. However, to simplify the following discussion, applicants will focus

⁵ Office Action (June 3, 2004), page 6, second paragraph.

⁶ Posewitz, page 2892, 2nd column, lines 4-7.

below on the first-listed unexpected benefit (enhanced luminescence instead of quenching), which alone should be sufficient for patentability.

II.D.i. The Posewitz Reference

Applicants strongly disagree with the Examiner about what Posewitz would have taught to one of ordinary skill in the art at the time the invention was made. The Examiner asserts that Posewitz teaches that gallium provides “more selective” retention of phosphorylated peptides, based on a speculative, unsupported statement near the end of the Posewitz reference (see Section II.C above). Applicants instead believe that one of skill in the art would have examined the data in Posewitz, rather than relying on this statement, to determine whether gallium provides more selective retention.

Posewitz compares purification of phosphopeptides using immobilized affinity chromatography (IMAC) with iron or gallium ions disposed in microtips. The purification involves two steps: (a) retention (binding) of phosphopeptide with iron or gallium in the microtips, and (b) elution (unbinding) of phosphopeptide from the iron or gallium with phosphate or base. The experiments performed by Posewitz do not determine whether retention or elution is more selective with gallium, because Posewitz does not measure the selectivity of retention and elution separately. For example, Posewitz does not measure how much nonphosphorylated peptide binds to iron and gallium in the microtips, independent from the amount that elutes. Furthermore, Posewitz shows that the phosphopeptide elutes (unbinds) from gallium more efficiently than from iron in the presence of base (see Figure 3), providing a teach away from the use of gallium in a binding assay, since one would not want phosphopeptide to unbind in a binding assay.

Posewitz does compare binding of a phosphopeptide (in the absence of nonphosphopeptides) to immobilized iron and gallium in microtips at two different pH values. The data in Figure 1 of Posewitz, reproduced here, show that comparable amounts of phosphopeptide (within statistical error) are retained by iron and gallium at each pH value, as indicated with asterisks (*) added by applicants.

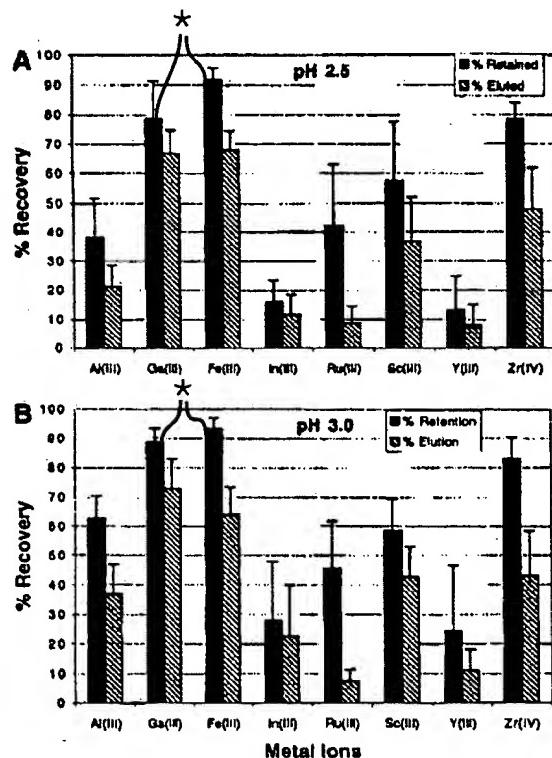


Figure 1 (Posewitz)

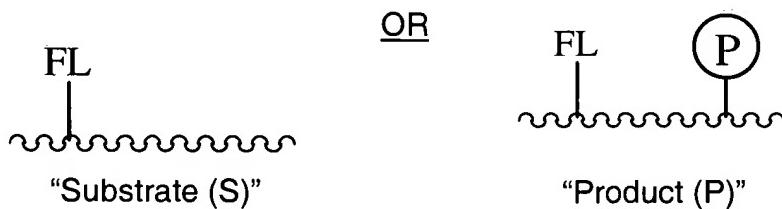
Accordingly, Posewitz shows that phosphopeptide binds with about the same efficiency to iron and gallium. This observation will be used for the analysis presented below in Subsection (ii).

Significantly, Posewitz does not teach or suggest any difference in the luminescence properties of iron and gallium (since Posewitz does not even mention luminescence at all). For example, Posewitz does not teach any difference in the ability

of iron and gallium to affect luminescence intensity when associated with a luminophore. Nevertheless, the Examiner has cited Posewitz's statement about the "unique features" of gallium to counter applicants' assertion of unexpected benefits of gallium over iron. These "unique features" relate to an alleged improvement in selectivity of gallium for retention of phosphopeptides. The "unique features" (i.e., the improved selectivity alleged by Posewitz) do not have any apparent relationship to luminescence properties and thus, independent of whether they are real or not, cannot and do not provide any expectation about the luminescence properties of either gallium or iron. Therefore, based on the teaching of Posewitz, one of skill in the art would not have expected gallium to show less (or more) quenching of luminescence intensity than iron when associated with a luminophore.

II.D.ii. Comparison of Actual and Expected Luminescence Intensities

Applicants previously presented a declaration with experimental data from binding assays comparing reagents that include iron ("iron reagent") or gallium ("gallium reagent").⁷ Each binding assay included either a fluorescent substrate (a luminophore-labeled, nonphosphorylated peptide; "S") or a fluorescent product (a phosphorylated form of the peptide; "P"), as follows:

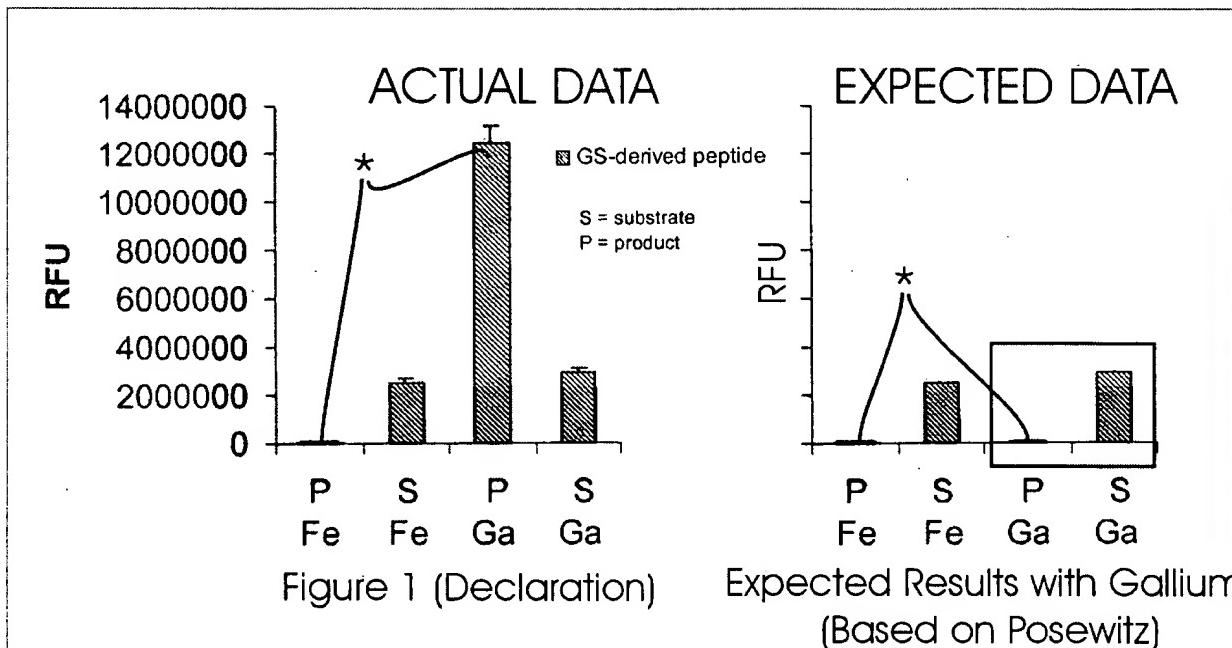


⁷ Response to Office Action (April 5, 2004), Exhibit A.

(The product corresponds to a product that would be produced by operation of a suitable kinase enzyme on the substrate.) The fluorescent substrate or product was incubated with the iron or gallium reagent. Then, the effect of each metal on luminescence intensity was measured. Accordingly, these binding assays with gallium correspond to an embodiment of the invention for detecting kinase enzyme activity in which there is no enzyme activity (substrate (S) only) or substantial enzyme activity (product (P) only).

Figure 1 of the declaration, which is reproduced below as the graph on the left, shows actual results of total luminescence intensity measurements, in relative fluorescence units (RFU), as a function of added metal (iron reagent or gallium reagent) and peptide phosphorylation state (P or S) in the binding assays. The luminescence intensity (or brightness) generally was comparable in assays of the substrate (S) performed with either the iron reagent or the gallium reagent. These results are consistent with little or no binding of each reagent to this nonphosphorylated substrate, and thus little or no effect on the intensity of luminophore associated with this substrate. In contrast, the luminescence intensity (or brightness) differed dramatically according to which of these reagents was present in assays of the product (P). In particular, the intensity was about one-hundred-fold higher in assays of the product performed with the gallium reagent relative to assays of the product performed with the iron reagent. This dramatic difference in intensity was produced, in part, by an approximately four-fold increase in the intensity of the product relative to substrate (S) for the gallium reagent, and, in part, by a more than twenty-fold decrease in the intensity of the product relative to substrate for the iron reagent. Therefore, in contrast to the iron reagent, which

quenched luminescence intensity substantially when present with product and its associated luminophore, the gallium reagent not only did not quench but actually enhanced this intensity. The increase in luminescence intensity provides substantial benefits to the claimed invention, as described further in Subsection (iii) below.



These actual results, obtained by applicants, are compared above with a graph of expected results for gallium, shown to the right of the actual results. (In the graph on the right, the actual results for iron from applicants are compared with the expected results for gallium (shown boxed), based on the prior art.) Posewitz shows comparable binding of a phosphopeptide to gallium and iron, and does not teach or suggest any difference between iron and gallium in the ability to affect luminescence intensity when bound to a luminophore-labeled material (see Subsection (i) above). Accordingly, one of skill in the art, without any information in the prior art to suggest otherwise, would have expected gallium to bind about the same amount as iron to the phosphopeptide, and thus to affect luminescence intensity about the same amount as iron when bound to

phosphopeptide. This expected result, marked above by an asterisk in the graph on the right, is dramatically different than the actual unexpected result from applicants, marked by the asterisk in the graph on the left. Furthermore, even if the Examiner asserts that one of skill in the art would have expected gallium and iron to have some measurable difference in their affect on luminescence intensity, there is no teaching or suggestion about the direction or magnitude of this difference.

The Examiner asserts that Posewitz teaches more selective binding to phosphopeptides. Applicants disagree, as described above in Subsection (i). However, even if Posewitz does teach more selective binding of phosphopeptides (relative to nonphosphopeptides) for gallium, this more selective binding would be manifested only as more binding of the nonphosphorylated peptide (substrate (S)) to iron, and less binding of this substrate to gallium (since Posewitz shows comparable binding of phosphopeptide to each of these metals). Furthermore, since both iron and gallium would be expected to affect luminescence intensity similarly when bound to luminophore-labeled substrate, less binding of the substrate to gallium might be expected to produce a greater luminescence intensity signal in the presence of gallium, due to less quenching provided by less binding. In contrast, applicants' actual results on the left show only a small difference in signal with substrate bound to gallium and iron, which may be produced by the opposite effects of gallium and iron on intensity when bound to luminophore-labeled peptide, as shown by applicants. Accordingly, applicants see little or no evidence of more selective binding by gallium. However, even a substantial increase in selective binding of gallium over iron to phosphopeptide (produced by a substantial difference in the amount of nonphosphopeptide bound by

each metal ion) would not have been expected to counteract the expected negative effect of substantial quenching of either metal when bound to luminophore-labeled product.

II.D.iii. Importance of the Unexpected Benefit of Increased Luminescence Intensity

The experimental results presented in the declaration⁸ and summarized above show that gallium provides a dramatic and unexpected increase in luminescence intensity relative to iron, when measured from metal-bound, luminophore-labeled peptide. Importantly, measurements of luminescence intensity underlie all measurements of luminescence polarization,⁹ so the increased luminescence intensity is an unexpected benefit of gallium in the claimed invention.

The dramatic differences in intensity between iron and gallium observed by applicants translate into dramatic differences in the timing and sensitivity of assays performed with these reagents. Polarization assays on large numbers of samples are configured to be analyzed as fast as possible, to maximize throughput. For example, the SmartRead™ system employed in polarization readers produced by Molecular Devices Corporation is designed to collect data on each sample until a threshold number of photons (or a timeout period) has been reached, and then move on to the next sample. This threshold number of photons typically is selected to correspond to a minimally

⁸ Id. at Exhibit A.

⁹ The relationship between polarization and intensity is expressed by the following equation:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Here, P is the polarization, I_{\parallel} is the intensity of luminescence polarized parallel to the polarization of the excitation light, and I_{\perp} is the intensity of luminescence polarized perpendicular to the polarization of the excitation light, all following excitation with polarized light. Thus, measurements of polarization are only as good as the underlying measurements of intensity. See, e.g., Application, page 52, lines 3-8.

acceptable signal-to-noise ratio for data analysis.¹⁰ Thus, due to the opposing effects of iron and gallium on brightness, it would take about 100 times as long to collect comparable light per sample with the iron reagent as with the gallium reagent. This difference literally is the difference between practical and impractical in high-throughput drug screening, since assay measurements that take 20-40 milliseconds to perform with gallium would take a completely unacceptable 2-4 seconds to perform with iron! Moreover, in any context, this difference means that for a given measurement time the signal strength (and thus the signal-to-noise ratio) will be significantly higher in gallium-based polarization assays than in iron-based polarization assays.

The dramatic intensity advantages of gallium relative to iron are not obvious. To the contrary, metals are well-known luminescence quenchers (i.e., extinguishers). Indeed, Pierce Biotechnology recently began selling a kinase assay system in which enzyme activity is observed using luminescence quenching that accompanies interaction of a fluorescently labeled phosphorylated peptide with iron.¹¹ Specifically, in the Pierce assay, luminescence intensity decreases monotonically with increasing phosphorylation, increasing kinase concentration, and increasing time, all reflecting increasing association of iron with the luminophore.¹² In contrast, applicants have discovered that gallium, unlike iron, not only does not quench but instead actually enhances intensity when bound to a luminophore. None of the references of record, including Nikiforov and Posewitz, teach or suggest this unexpected and patentable benefit of using a binding partner including gallium in polarization assays.

¹⁰ The signal-to-noise ratio of the intensity in photon processes is proportional to the square root of the number of photons collected.

¹¹ See, e.g., Pierce IQ® Assay Platform: Technical Handbook (Pierce Pub. No. 1600963) (August 2003). This handbook is included with the Response to Office Action (April 5, 2004) as Exhibit B.

¹² Id.

In summary, the unexpected benefit of increased luminescence intensity provided by gallium far outweighs any expected benefit of gallium in the claimed invention that might be derived from the teachings of Posewitz. In particular, without this unexpected benefit, any expected benefit from increased selectivity of gallium allegedly taught by Posewitz would still be dominated by the expected quenching by gallium. The lack of quenching by gallium, as shown by applicants, which provides an unexpected benefit of increased luminescence, thus both enables the claimed assay and rebuts any assertion of *prima facie* obviousness by the Examiner. Therefore, for at least these reasons, applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 103(a) and prompt allowance of the pending claims.

III. Conclusion

Applicants believe that they have addressed all of the issues raised by the Examiner in the Office action, and that the application currently is in condition for allowance. However, if the Examiner has any questions or comments, or if a telephone interview would advance the prosecution of the application, the Examiner is encouraged to call applicants' undersigned attorney at the telephone number listed below.

CERTIFICATE OF MAILING

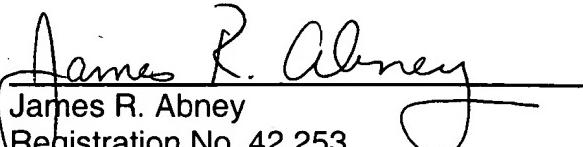
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: Mail Stop AMENDMENT, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on December 3, 2004.



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